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Ring-Opening Metathesis Polymerization-Derived Monolithic Materials: Novel Syntheses and Applications

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Summary: Selected recent accomplishments in the ring-opening metathesis polymerization-based synthesis of polymeric monolithic materials are summarized. Both synthetic and applicative aspects are included.

Keywords: biomaterials; catalysis; chromatography; molding; ring-opening metathesis polymerization; synthesis

Introduction

Monolithic polymeric materials have first been reported in the early 1990s[1,2] and have nowadays become an integral part of polymer chemistry and material science. They are characterized by a unitary porous structure with interconnected large pores and are usually synthesized within the confines of the compartment in which they are to be used at a later stage. This avoids any packing procedures. The structure-forming matrix composed of interlinked microglobules itself may be non-porous or porous, depending on the application. Particularly monoliths based on non-porous structure-forming microglobules allow for the separation of medium and high molecular weight analytes such as proteins, peptides and ologonucleotides within very short times of analysis, typically within less than 60 seconds.[3] This is related to the fact that there is no diffusion of analytes into small pores. Instead, the entire interaction of the analytes with the stationary phase occurs at the non-porous inner surface of the monolith, giving resulting in fast mass transfer between the stationary and mobile phase.

So far, the majority of monolithic polymeric materials are prepared via thermally or UV-induced free radical polymerization of (meth)acrylates,[3,4] however, other techniques such as γ-irradiation or electron beam triggered free radical polymerization as well as various polyaddition and polycondensation based approaches have to be mentioned, too. Apart from these synthetic approaches, the ring-opening metathesis polymerization (ROMP) based synthesis and functionalization of such supports has been elaborated by our group[5,6] aiming at applications in separation science,[7–17] heterogeneous catalysis[18–23] and tissue engineering.[24,25] Here, the most recent accomplishments in the synthesis of ROMP-derived monoliths as well as selected applications are summarized.

Results and Discussion

Schrock Initiator-Derived Monoliths[9]

In order to be compatible with Schrock type initiators, the novel polymerization system did not contain any protic

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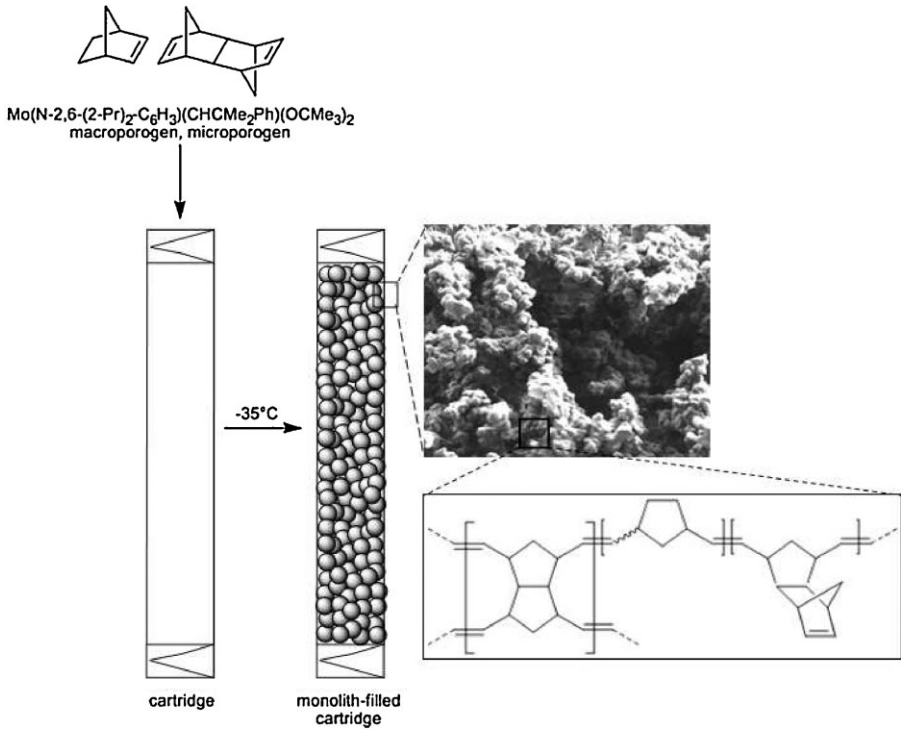
compounds. It consisted of norborn-2-ene (NBE), 1,4,4a,5,8,8a-hexahydro-1,4,5,8-*exo, endo*-dimethanonaphthalene (DMN-H6), a microporogen, e.g., 1,2-dichloroethane, toluene or THF, and a macroporogen, i.e. hexane or pentane, as well as of the initiator, i.e. Mo(N-2,6-(2-Pr)$_2$-C$_6$H$_3$)(CHCMe$_2$Ph)(OCMe$_3$)$_2$ (**1**). The reaction is illustrated in Scheme 1. Using different amounts of monomer, cross linker, and porogens, a mixture composed of NBE: DMN-H6:1,2-dichloroethane:hexane:**1** = 15: 15:10:60:0.1 was found to be best suited for the separation of low molecular weight analytes.

The living sites were terminated by passing a 3 wt.-% solution of ferrocene carbaldehyde in THF through the monolith. This simple Wittig-type reaction between the living polymer chains and an aldehyde does not only remove the initiator, but also provides a very simple and elegant way for in situ functionalization. Thus, the nature of the functional group is basically only limited by the functionality of the corresponding aldehyde.

Monolithic Materials for Tissue Engineering[24,25]

There is a strong need for soft tissue augmentation in various surgical fields for adipose engineering. Beside its essential metabolic functions, adipose tissue provides the shape and volume of the outer contour of the body and preserves the mobility of tissue layers. Soft tissue augmentation is thus necessary in case of congenital disorders, after tumor ablation as well as in esthetic or reconstructive surgery. Due to its high metabolic activity, however, adipose tissue cannot be transplanted freely in larger amounts and needs vascular supply.[26] adipose tissue



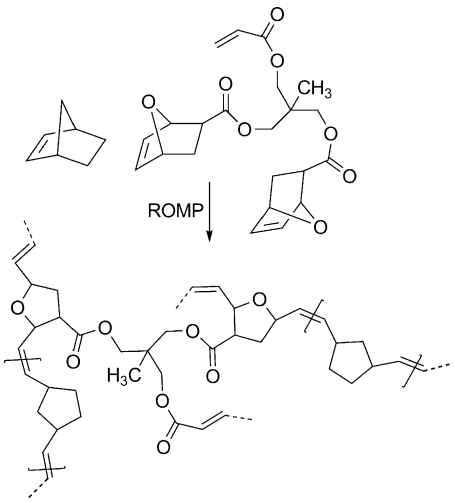
Scheme 1.
Schrock initiator triggered synthesis of monolithic materials.

engineering might is thus considered to enlarge the potential and the versatility of autologous soft tissue augmentation. The advantages over existing methods are: a) persistent augmentation after single or few injections, b) no side effects due to sensitizing agents, c) possibility for later corrections, d) cost-effectiveness as compared with current dermal filers.

In search of alternative scaffolds suitable for tissue engineering, we focused on monolithic supports. The molding processes that are feasible significantly reduce restrictions in shape. We developed a ROMP-based protocol based on highly hydrophilic monomers. Thus, by using a 20:20 wt.-% mixture of norborn-2-ene (NBE) and pentaglycerol bis(7-oxanorborn-5- ene-2-ylcarboxylate) acrylate (PGBA) in a microporogen (toluene) and a macroporogen (2-propanol, 5:10 wt.-% ratio), monolithic structures were realized with the aid of $RuCl_2(pyridine)_2(IMesH_2)$ (CHPh) (0.03 wt-%) and low amounts of additional pyridine as regulator.[24] Scheme 2 illustrates the process of monolith synthesis; a typical structure with pores up to 400 μm is shown in Figure 1.

In view of any intoxicating effects of catalyst residues, a careful washing of the monolithic structure with a mixture of dimethylsulfoxide, ethyl vinyl ether and THF was carried out and allowed for the virtually quantitative removal of the transition metal-based initiator, resulting in final ruthenium concentrations <0.1 ppm (i.e. below the limit of detection of inductively coupled plasma optical emission spectroscopy). Upon storage in a buffer system, the initial water contact angle of 142° rapidly decreased within 13 weeks. In fact, no reliable values could be determined due to fast moistening, i.e. spreading. This is attributed to both the highly hydrophilic character of PGBA and the high propensity towards oxidation of the poly(NBE) blocks, which experience oxidation in allylic position.

Then the ROMP-derived scaffolds were cultivated with adipose tissue stem cells (ATSCs). Within 12 days, the number of cells quadruplated at least at the surface. No additional compounds such as fibronectine, RGD-peptides or matrix factors, factors (aside from differentiation factors or supplements) commonly used to facilitate or trigger cell growth, were necessary. In order to study cell ingrowth and differentiation on the scaffolds in a three-dimensional setting, cultivation was performed under dynamic conditions in rotating culture containers. Important enough, cells grew *into* the pores and partially showed even full ingrowth and penetration of the scaffold. Finally, after growth, these cells were then successfully subjected to both osteogenic and adipocytic differentiation. Differentiation of ATSCs into either adipocytes or osteoblasts was initiated by addition of isobutylmethylxanthine or ß-glycerolphosphate and dexamethasone, respectively. Figure 2A shows adipocytes as identified by the formation of large univacuolar cells. Figure 2B shows mineralizations by the cells grown *within* the monolithic material after 6 weeks. As can be seen, biomineralization was effective with these materials.



Scheme 2.
7-Oxanorborn-2-ene-based, ROMP-derived monoliths.

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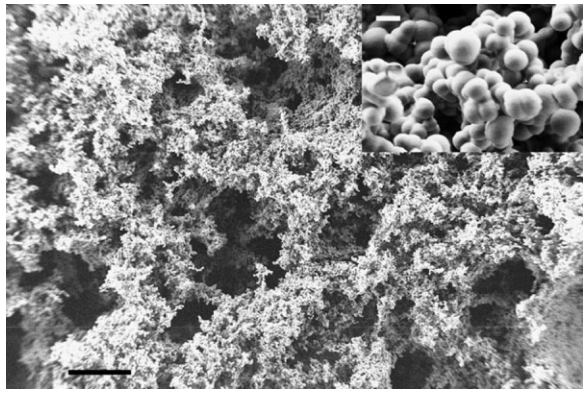

Figure 1.
Porous, ROMP-derived monolithic scaffolds. The scale bar represents 400 μm. The scale bar in the right insert corresponds to 4 μm.

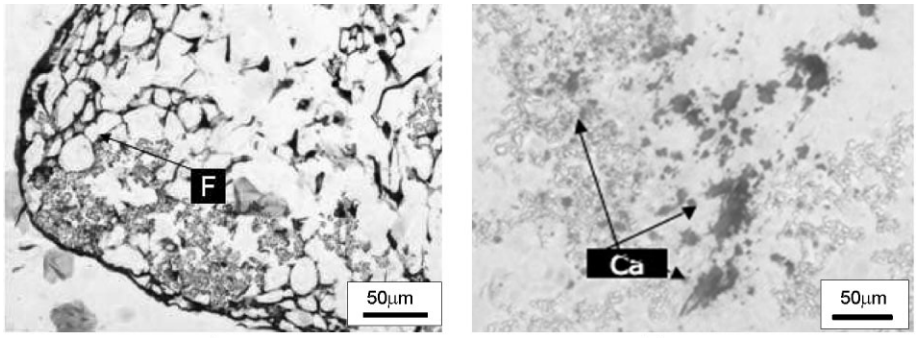

Figure 2.
Histomicrographs of monoliths seeded with adipose tissue derived stem cells after adipogenic (left) and osteogenic differentiation (right). A: Low power magnification of scaffold cross-section with cell ingrowth between the scaffold material after adipogenic differentiation. Fat cells with nuclei ("**F**") are visible also in the center of the cross section of the scaffold (HE). B: Von Kossa stain shows massive mineralizations (arrows, black-brown) after osteogenic differentiation within the monolithic scaffold (counterstaining of cell nuclei with HE). The scaffold material is invaded by cellular ingrowth.

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[1] B. G. Belenkii, A. M. Podkladenko, O. I. Kurenbin, V. G. Mal'tsev, D. G. Nasledov, S. A. Trushin, *J. Chromatogr.* **1993**, *645*, 1–15.
[2] V. G. Mal'tsev, D. G. Nasledov, S. A. Trushin, T. B. Tennikova, S. V. Vinogradova, I. N. Volokitina, V. N. Zgonnik, B. G. Belenkii, *J. High Resolut. Chromatogr.* **1990**, *13*, 185–189.
[3] F. Švec, T. B. Tennikova, Z. Deyl, *Monolithic Materials: Preparation, Properties and Application*, Elsevier, Amsterdam, 2003.
[4] M. R. Buchmeiser, *Polymer* **2007**, *48*, 2187–2198.
[5] F. Sinner, M. R. Buchmeiser, *Angew. Chem.* **2000**, *112*, 1491–1494.
[6] F. Sinner, M. R. Buchmeiser, *Macromolecules* **2000**, *33*, 5777–5786.
[7] R. Bandari, A. Prager-Duschke, C. Kühnel, U. Decker, B. Schlemmer, M. R. Buchmeiser, *Macromolecules* **2006**, *39*, 5222–5229.
[8] R. Bandari, W. Knolle, M. R. Buchmeiser, *J. Chromatogr. A.* **2008**, *1191*, 268–273.
[9] B. Scheibitz, A. Prager, M. R. Buchmeiser, *Macromolecules* **2009**, *42*, 3493–3499.
[10] B. Mayr, R. Tessadri, E. Post, M. R. Buchmeiser, *Anal. Chem.* **2001**, *73*, 4071–4078.
[11] M. R. Buchmeiser, *Macromol. Rapid. Commun.* **2001**, *22*, 1081–1094.
[12] S. Lubbad, B. Mayr, C. G. Huber, M. R. Buchmeiser, *J. Chromatogr. A* **2002**, *959*, 121–129.

[13] S. Lubbad, M. R. Buchmeiser, *Macromol. Rapid Commun.* **2002**, *23*, 617–621.
[14] S. Lubbad, S. A. Steiner, J. S. Fritz, M. R. Buchmeiser, *J. Chromatogr. A* **2006**, *1109*, 86–91.
[15] C. Gatschelhofer, C. Magnes, T. R. Pieber, M. R. Buchmeiser, F. M. Sinner, *J. Chromatogr. A* **2005**, *1090*, 81–89.
[16] B. Schlemmer, G. Gatschelhofer, T. R. Pieber, F. M. Sinner, M. R. Buchmeiser, *J. Chromatogr. A* **2006**, *1132*, 124–131.
[17] F. M. Sinner, C. Gatschelhofer, A. Mautner, C. Magnes, M. R. Buchmeiser, T. R. Pieber, *J. Chromatogr. A.* **2008**, *1191*, 274–281.
[18] M. Mayr, B. Mayr, M. R. Buchmeiser, *Angew. Chem.* **2001**, *113*, 3957–3960.
[19] M. R. Buchmeiser, S. Lubbad, M. Mayr, K. Wurst, *Inorg. Chim. Acta* **2003**, *345*, 145–153.
[20] J. O. Krause, S. Lubbad, O. Nuyken, M. R. Buchmeiser, *Adv. Synth. Catal.* **2003**, *345*, 996–1004.
[21] J. O. Krause, S. H. Lubbad, O. Nuyken, M. R. Buchmeiser, *Macromol. Rapid Commun.* **2003**, *24*, 875–878.
[22] M. Mayr, D. Wang, R. Kröll, N. Schuler, S. Prühs, A. Fürstner, M. R. Buchmeiser, *Adv. Synth. Catal.* **2005**, *347*, 484–492.
[23] M. J. Beier, W. Knolle, A. Prager-Duschke, M. R. Buchmeiser, *Macromol. Rapid Commun.* **2008**, *29*, 904–908.
[24] A. Löber, A. Verch, B. Schlemmer, S. Höfer, B. Frerich, M. R. Buchmeiser, *Angew. Chem.* **2008**, *120*, 9279–9281.
[25] M. R. Buchmeiser, *J. Polym. Sci. A: Polym. Chem.* **2009**, *27*, 2219–2227.
[26] J. Smahel, *Ann. Plastic Surg.* **1986**, *16*, 444–453.

